https://doi.org/10.17221/111/2019-CJGPB

Phenotypic traits for wild red clover seed yield under drought conditions

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Citation: Petrauskas G., Norkevičienė E., Stukonis V., Kemešytė V. (2020): Phenotypic traits for wild red clover seed yield under drought conditions. Czech J. Genet. Plant Breed., 56: 140–149.

Abstract: Changes in the gene pool and homogeneity of red clover cultivars occur over time. Therefore, it is necessary to constantly renew the breeding material and foundation seed. Moreover, the market also prescribes a new demand. Based on the Natura 2000 habitats profile, we collected seeds from 39 locations across Lithuania in 2016. The phenotypic traits that affect the seed yield of red clover were analysed in 2018. The homogeneity of the populations with reference to the seed yield and 1 000 seed weight (TSW) were determined as well. Also, the possibility of obtaining two seed yields per season under drought conditions from wild genotypes of red clover was analysed. We found that the final seed yield mostly depends on the seed number per flower head (SN/FH), which strongly correlated with 1st component of PCA during first (r = 0.91) and second (0.92) harvest. Meanwhile, the cluster analysis showed that the typical wild red clover has a lower seed weight than the cultivars and could be clustered on the basis of seed homogeneity. Finally, based on the seed phenotype and harvest components, there were five prospective accessions (2177, 2871, 2876, 2898 and 2899) for a new cultivar prototype.

Keywords: autochthonous clover; G × E interaction; yield determination

The fodder value of the red clover (*Trifolium pratense* L.) has been known for centuries. It has been reported that red clover has been cultivated from the 16th century onward in some parts of Europe (Mousset-Dèclas 1995; Annicchiarico et al. 2015). For years, it was used to feed livestock, mostly cattle. However, in the last decade, it has been reported that cattle herds are not growing, but shrinking in the European Union, because of animal welfare issues, the environmental impact and pasture availability (Smith et al. 2018). This has not been matched by a decrease in the demand for red clover. Moreover, the demand for red clover cultivars is growing in Asia, where a supply is needed to meet the farmers' demands (Boelt et al. 2015). Farmers are interested in cultivars that are more adapted to specific agro-climatic conditions in particular growing zones (Smith et al. 2018). Moreover, the demand for red clover cultivars for specific purposes has increased. Unlike alsike clover (*Trifolium hybridum* L.), red clover (*Trifolium pratense* L.) has a lower content of tannins, saponins and other biologically active substances that are not particularly suitable for animal feed (Butkutė et al. 2018, 2019). Even so, it has enough secondary metabolites and bioactive substances to make red clover attractive as a supplementary food product or a clinical therapeutic agent (Lemežienė et al. 2015; Butkutė et al. 2018, 2019). On the other hand, researchers have focused only on the feed value of red clover and the benefits of enriching the soil

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Supported by the long-term research programme "Genetics and purposeful change of genotypes of agricultural and forest plants" implemented by the Lithuanian Research Centre for Agriculture and Forestry.

with atmospheric nitrogen (Dewhurst et al. 2009). Breeding red clover for ornamental purposes and beekeeping has not been extensively studied.

It is known that wild forms of red clover have a relatively strong differentiation from their domesticated relatives. New cultivars could be characterised by a large number of dark pink inflorescences, a high number of flowers per flower head, an early flowering and biological potential to produce two seed yields per season (Annicchiarico *et al*. 2015; Taylor & Quesenberry 1996). Breeding for such agronomic purposes has not been carried out in Lithuania. Also, no publication was found detailing the possibility of breeding a diploid red clover cultivar in Lithuania. A survey of local farmers, ornamental plant growers and beekeepers has shown that a red clover cultivar of such characteristics would be in demand in Lithuania. Wild red clover is known to be a starting point for subsequent breeding programmes. The initial material should be found in natural habitats with a high biodiversity and a low level of anthropogenic activity (Boller et al. 2010; Petrauskas et al. 2018). In this study, traits of wild diploid red clovers have been analysed to gain a better understanding of the seed yield production under drought conditions. The agro-biological potential of producing two seed yields per season has also been analysed. Usually, the seed yield, phenotypic traits and feed value are the key indicators of a good breeding material for a new red clover cultivar. However, the indicators, such as the feed value or biomass, would not be so relevant for the development of an ornamental cultivar. Still, the period and duration of the flowering in breeding red clover cultivars for beekeeping could be considered.

MATERIAL AND METHODS

Seeds from wild populations inventoried as Natura 2000 habitats (codes 6510, 6530, 6270, 6210) were collected across Lithuania in 2016. More than 60 different locations were visited, but some grasslands had been cut down or ploughed over; therefore, seeds were collected from 39 populations in total.

The study involved six locally bred cultivars (Liepsna, Kiršinai, Radviliai, Sadūnai, Vytis, Vyliai) and four received from other countries' plant gene banks (Renova, Temara, Barfiola and Tempus, respectively marked as 2887, 2888, 2890 and 2908). The local bred cultivar Vytis was chosen as the standard. This cultivar is characterised by its early flowering and high seed productivity.

The research was carried out during the 2017–2018 season. Red clover was sown and germinated in palettes filled with a peat and soil mixture (30 : 70) in the greenhouse on 18 April 2017. During growth, the plants were watered every day and were cut twice before being transplanted to the field trial. Three months later, the plants were moved into an experimental field *ex situ* on 10 July 2017. The seed yield studies were performed during 2018. The meteorological conditions, such as air temperature and precipitation, were assessed in 2018. The soil matric potential was recorded from Watermark (WM) probes at 20, 50 and 100 cm depths. The study area is located at 55°23'35.0''N 23°52'38.6''E. Based on the Lithuanian soil classification (LTDK-99), it is an Endocalcari–Epihypogleyic Cambisol (RDg4-k2) soil with a moderately heavy loam texture predominates or a Gleyic Cambisol according to the WRB 2014 update 2015 (IUSS Working Group WRB 2015). The field trial was arranged in 4 replicates in randomised plots 1 × 5 m with fifteen plants per plot. Ten flower heads were randomly selected from ten plants per each accession for the seed yield determination. The number of total flower heads and florets per plant were counted. The thousand-seed weight was determined, as well as the seed set, and the germination rate was evaluated following the international rules

Table 1. The phenotypic traits used in the study and their abbreviations

Phenotypic trait description	Abbreviation	Determination method
Flower heads per plant (pcs)	FH	direct counting
Florets per flower head (pcs)	F/FH	direct counting
Seed number per flower head (pcs)	SN/FH	direct counting
Seed set (%)	SS	
Germination rate (%)	GR	direct counting percentage
Hard seeds (%)	HS	direct counting percentage
Thousand seed weight (g)	TSW	direct weighing
Total seed yield per 100 head (g)	SY	direct weighing

for seed testing (ISTA). The percentage of hard seeds in each sample was estimated. A study of the seed yield was performed individually for each accession during the first and second maturation periods in the same season.

No fertiliser was used in the field trial during the study. Weeds were controlled mechanically and by the active agent bentazone 480 g/l (commercial herbicide Basagran 480 with a dose of 960 g/ha) twice per season.

Statistical analyses. The statistical analyses were performed with the software SAS 9.4 (SAS Institute Inc., USA). Graphs were visualised by using software Veusz (Ver. 3.2.1, 2020). The abbreviations of scored red clover characteristics are shown in Table 1.

RESULTS

It is known that tetraploids of red clover produce fewer, but heavier, seeds than diploids (Taylor & Quesenberry 1996; Vleugels et al. 2016). In this study, the seed yield of wild red clover populations was analysed in comparison to diploid and tetraploid cultivars. A 2D cluster analysis of red clover seed homogeneity was conducted on the basis of the seed yield (SY) per accession and the 1 000 seed weight (TSW) (Figure 1). The variables in the red clover accessions were classified by the colour analysis variable SY and the size analysis variable TSW. Based on the 2D cluster analysis, the cell size represented the TSW, with larger cells having a higher TSW. This assumes that larger seeds are most likely tetraploid. The tetraploid cultivars 2888, 2890, 2908 and Kiršinai are clustered in the same group on the basis of the cell size according to the TSW, but the total seed yield per accession is low based on the colour intensity, which is specific for tetraploids. However, the tetraploid cultivars Sadūnai and Vyliai, did not show such a high TSW, unlike the other tetraploids. Those two cultivars displayed a fairly high yield, so it is likely that they are not completely homogeneous and may be mixed with diploids. The total SY per accession (5.37 g) of both harvests was the lowest for the tetraploid cultivar 2890, and a similar yield of 5.98, 6.72 and 5.98 g was obtained in the other tetraploid cultivars 2888, 2908 and Kiršinai, respectively. However, the cultivars (2888, 2890, 2908 and Kiršinai) were distinguished by the highest TSW of 2.595, 2.685, 2.84 and 2.095 g, respectively. The largest SY of both harvests among the wild populations was identified from accessions 2898, 2899, and 2177 (24.39, 23.77 and 24.45 g, respectively). Among these three populations, the highest TSW was found in

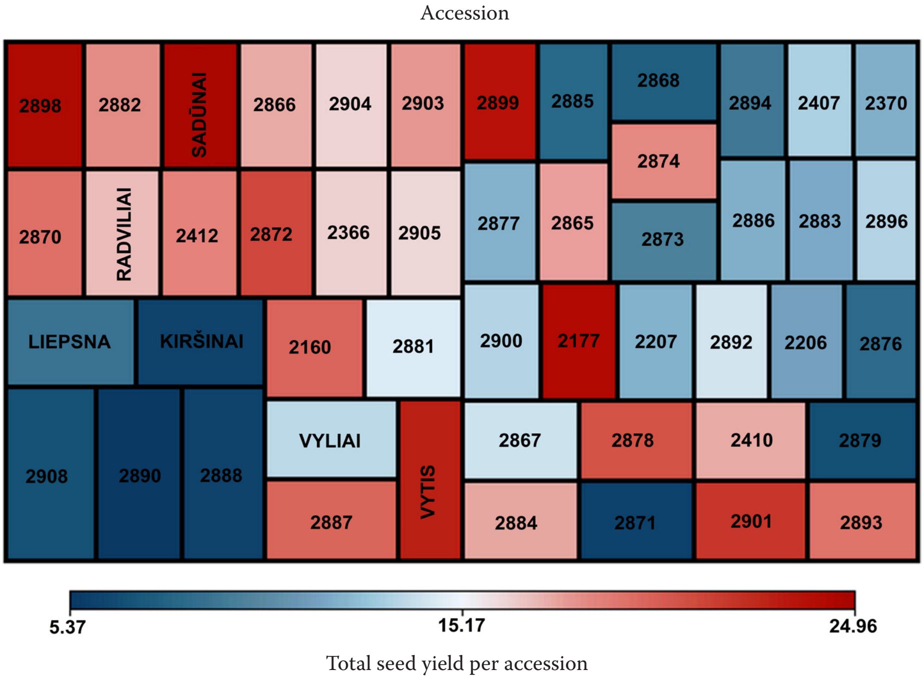

Figure 1. A 2D cluster analysis of the red clover seeds homogeneity; the tile chart represents values of two different categories – the total seed yield from 100 flower heads per accession (in g) (square colour) and the 1 000 seed weight (TSW in g) (square size)

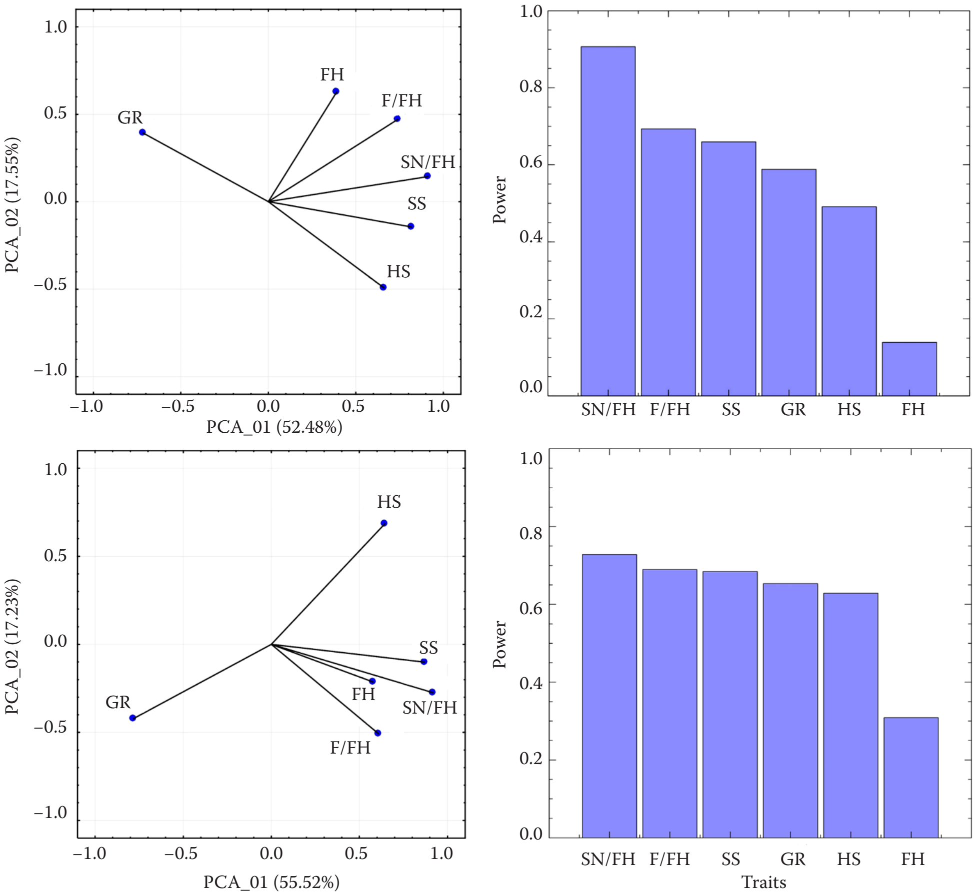

Figure 2. The principal component analysis (PCA) based on the important traits for the seed yield and power columns of the traits importance in the data set

FH – flower heads per plant; F/FH – florets per flower head; SS – seed set; SN/FH – No. of seeds per flower head; GR – germination rate; HS – hard seeds

accession 2898 (1.8 g). Meanwhile, the lowest TSW was found in accession 2370 (1.37 g) collected in the wild ecotype. The lowest total seed yield was found in accession 2871 (5.83 g) and 2879 (6.57 g). The standard cultivar Vytis produced a total SY of 23.02 g and a TSW of 1.9 g.

The components of the first seed harvest were evaluated. The first component explained 52.48% of the cases in the variance in the total seed set (Figure 2). While, the second component explains 17.55% of the cases, and the remaining 29.97% of the cases are explained by components 3–6. The highest positive correlation ($r = 0.91$) was identified between the first component and the number of seeds per flower head (SN/FH), while a negative correlation between the first component and the germination rate (GR) was found ($r = –0.72$). Meanwhile, focusing on the second component, the highest positive correlation was found between this component and the number of FH ($r = 0.63$), although the highest negative correlation was identified between the first component and the germination rate. This tendency was different for the second component. The highest negative correlation was found in the hard seeds ($r = –0.49$).

Summarising the results, the most important indicator was the SN/FH according to the first component and the FH according to the second component, but since the first component highlights a higher number of cases, it is considered more important for the seed yield prediction. This was confirmed by the power ranking, which was close to 0.90 (0.906822).

Meanwhile, 55.52% of the second seed harvest is explained by the first component and 17.23% by the second, while the remaining 27.25% of the cases are explained by components 3–6 of the entire data set. Analysing the first component of the second harvest, it was found that the SN/FH had the strongest positive correlation with the first component ($r = 0.92$). The highest positive correlation of the second component was found in the hard seeds (HS) ($r = 0.68$),

https://doi.org/10.17221/111/2019-CJGPB

Table 2. The mean values and standard errors for six phenotypic traits on the first harvest in 2018

Accession	FH	F/FH	SN/FH	SS	GR	HS
		(pcs)			(%)	
Vytis	54 ± 2.6	113 ± 5.7	72 ± 2.9	64 ± 2.5	19.5 ± 0.6	79.75 ± 1.6
2160	69	83** ± 3.4	49** ± 2.2	59	2.25** ± 0.3	97.75
2177	123* ± 5.3	132	62	47** ± 1.7	12.25** ± 0.9	86.25
2206	71	81** ± 2.6	37** ± 1.6	46** ± 2.3	2** ± 0.4	94.25* ± 3.1
2207	79	84** ± 3.6	45** ± 3.1	54	20.25	75.75
2366	112* ± 6.8	112	65	58	12** ± 0.4	82.25
2370	68	119	61	51	14.25** ± 0.9	0** ± 0
2407	70	114	52** ± 3.4	46** ± 3.4	0** ± 0	100* ± 0
2410	92* ± 9.3	121	76	63	9.75** ± 0.5	86.25
2412	73	116	73	63	16.25	81.75
2865	81* ± 5.2	111	65	59	8** ± 0.7	92* ± 3.1
2866	69	90	58** ± 3.5	64	2.25** ± 0.3	95.75* ± 2.8
2867	77	63** ± 5.1	39** ± 2.8	62	13.75** ± 0.6	85.75
2868	98* ± 5.4	86** ± 7.1	23** ± 1.0	27** ± 3.5	8.25** ± 0.5	72.25
2870	94* ± 4.5	92	54** ± 3.1	59	17.75	81.75
2871	104* ± 5.4	81** ± 3.4	18** ± 0.7	22** ± 2.8	10** ± 0.6	82
2872	88* ± 7.5	85** ± 5.0	50** ± 2.7	59	21.75	71.5
2873	89* ± 9.6	94	36** ± 1.3	38** ± 2.2	18.25	81.75
2874	127* ± 4.8	110	74	61	14.5** ± 1.2	84.25
2876	89* ± 4.6	83** ± 4.8	31** ± 1.6	37** ± 4.1	27.75** ± 1.3	74
2877	68	72** ± 5.3	29** ± 1.1	40** ± 2.5	4** ± 0.4	95.75* ± 1.9
2878	60	88	54** ± 3.9	61	7.5** ± 0.3	91.75* ± 1.9
2879	101* ± 11.4	96	16** ± 0.8	17** ± 1.1	36.25* ± 1.9	61.75** ± 3.0
2881	43	99	52** ± 4.3	53	12** ± 0.4	88.25
2882	98* ± 4.5	107	50** ± 2.4	47** ± 2.9	16.25	80
2883	121* ± 10.1	101	51** ± 3.6	51	2** ± 0.4	97.5* ± 1.6
2884	53	87** ± 5.1	47** ± 2.7	54	1.75** ± 0.6	79.75
2885	94* ± 6.9	88** ± 3.9	22** ± 0.9	25** ± 2.4	2.5** ± 1.0	79.75
2886	75	92	36** ± 0.9	39** ± 2.8	8** ± 1.2	86.25
2887	38	90	62	69	41.5* ± 1.8	55.75** ± 3.9
2888	84	79** ± 4.5	18** ± 1.0	23** ± 1.6	46* ± 0.9	54** ± 2.1
2890	37	63** ± 2.7	12** ± 0.4	19** ± 0.8	50* ± 2.0	48** ± 3.6
2892	75	94	58** ± 3.4	62	13.75** ± 0.6	83.75
2893	111* ± 7.8	94	59** ± 4.2	63	1.75** ± 0.5	97.3* ± 1.6
2894	94* ± 6.5	71** ± 5.3	32** ± 2.1	45** ± 3.3	6.25** ± 0.6	8** ± 0.9
2896	116* ± 3.8	110	55** ± 2.7	50	19.75	76.25
2898	95* ± 8.1	86** ± 4.6	54** ± 1.8	63	23.75	76
2899	115* ± 8.4	122	85* ± 3.2	70	2.25** ± 0.6	95.75* ± 1.5
2900	96* ± 7.0	98	41** ± 2.3	42** ± 4.0	16	47.5** ± 4.3
2901	115* ± 6.1	114	74	65	11.75** ± 0.8	86.25
2903	111* ± 7.2	98	55** ± 2.6	56	14.25** ± 0.5	100* ± 0
2904	58	112	49** ± 3.0	44** ± 1.8	25.75* ± 0.9	73.75
2905	90* ± 6.4	98	54** ± 3.2	55	23.75	75.75
2908	39	65** ± 2.6	15** ± 0.6	23** ± 1.0	60* ± 3.1	38.25** ± 2.0
Kiršinai	99* ± 4.5	70** ± 6.2	19** ± 1.2	27** ± 2.3	84.75* ± 2.7	5.75** ± 1.3
Liepsna	60	61** ± 6.9	23** ± 1.6	38** ± 1.8	29.5* ± 0.9	70.25
Radviliai	99* ± 4.5	115	62	54	17.75	79.75
Sadūnai	78	123	44** ± 3.5	36** ± 1.8	30* ± 1.1	67.75** ± 2.9
Vyliai	89* ± 8.7	94	41** ± 2.2	44** ± 2.7	39.75* ± 1.1	50** ± 1.9

*significantly higher than the standard Vytis $P \leq 0.05$ (Dunnett's test); **significantly lower than the standard Vytis $P \leq 0.05$ (Dunnett's test); FH – flower heads per plant; F/FH – florets per flower head; SS – seed set; GR – germination rate; HS – hard seeds and all the other traits negatively correlated with the second component.

The phenotypic traits ranked by importance were in the same order as for the first harvest, but the power ranking differed slightly (from 0.63 to 0.73), except for the FH, which had a power of 0.31 compared with 0.13 in the first harvest.

The statistical analysis based on Dunnett's test shows that the different phenotypic traits of the flowers and seeds differed between the first and second seed yields (Table 2). In the first harvest, 27 accessions were significantly different with respect to the FH compared with the standard cultivar Vytis ($P \leq 0.05$), and 21 accessions did not differ significantly. The number of florets per flower head (F/FH) showed the opposite trend: 18 accessions were significantly lower than Vytis, and 29 accessions did not show any significant differences. The SN/FH was significantly lower in almost all of the accessions than in the Vytis cultivar, and only 10 accessions did not differ significantly. There was a similar trend in the seed set (SS), but the higher number of accessions (26) did not differ significantly from the Vytis cultivar. Meanwhile, the GR showed that 10 accessions were significantly higher, 28 were significantly lower and 10 were not significantly different compared to the cultivar Vytis. Ten accessions had significantly higher rates of hard seeds and 11 had significantly lower rates than the Vytis cultivar.

The second seed yield was dominated by a high variability in the phenotypic differences among the populations (Table 3). It is most likely that not all the populations are adapted for two cuts. The water deficit affected the regrowth time, seed formation and other productive elements. The effect of the water deficit varied in the first and second harvest among the populations.

According to the literature, the limit of water available to most of plants is considered to be from the field capacity of 10 kPa (0.1 bar) to the permanent wilting point (PWP) of 1 500 kPa (15 bar) (Bhattacharya 2019). Meanwhile, plants sustain drought stress when the tension raises to 100 kPa (1 bar) (Bhattacharya 2019). Our measurements showed a water deficit at a depth of 20 cm from mid-May to mid-June (Figure 3). The second wave of water deficit ran up from August till the end of measurements on October 20. At a depth of 50 cm, the water deficit was recorded from mid-June to the 10th of July and once again from the 20th of July to the end of the measurements. Meanwhile, at the depth of 100 cm, a water deficit occurred only once from mid-August till the end of the experiment. This indicates that the red clover was under drought stress during all the vegetative periods except for a short period in July, when the plants were re-growing after the first cut. New inflorescences were formed in July depending on the accession. The time of flowering lasted about 4–6 weeks. During the plant vegetation period, the vegetation was mostly very dry and hot (Figure 4). The average temperature in April was 3 °C higher than the climatological norm (CN) of 6.9 °C and reached 9.9 °C. Meanwhile, the precipitation was 52.2 mm (CN 37 mm). May was very hot, sunny and extremely dry. The average temperature was 16.9 °C, while the CN was 12.4 °C. This month precipitation quantity reached 76.9% of the CN (52.3 mm). The summer was very hot and dry as well. The average temperature in June was 1.6 °C higher than the CN (15.9 °C), while the precipitation was 44.9 % lower than the CN (61.9 mm). The plants were stressed due to the water deficit and the vegetation sharply reduced. A severe drought was proclaimed by the Lithuanian government in June. In the meantime, July started with windy and rainy weather, but later, the temperature rose to be even higher than 30 °C. Despite the fact that the precipitation of this month reached 144.9% of the climatological norm (57.5 mm), evaporation was intense and the soil moisture was insufficient at the end of the month. August was

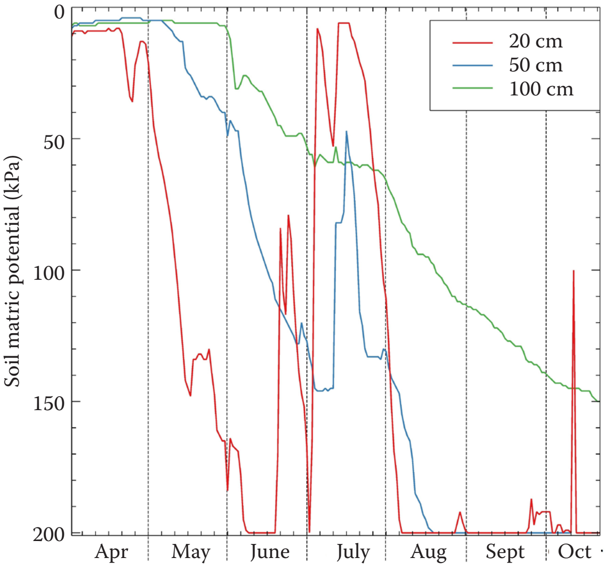

Figure 3. The daily soil matric potentials from the watermark sensors at 20, 50 and 100 cm soil depths during the 2018 season

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Table 3. The mean values and standard errors for six phenotypic traits on the second harvest in 2018

Accession	FH	F/FH	SN/FH	SS	GR	HS
	(pcs)			(%)		
Vytis	65.5 ± 2.8	112 ± 4.9	45 ± 1.5	40 ± 2.9	36 ± 1.7	44.25 ± 2.2
2160	88.3	91	50	55* ± 5.1	20** ± 0.9	72* ± 1.5
2177	72.3	98	51	52	14** ± 0.6	74* ± 1.6
2206	61.1	89	33** ± 2.2	37	20.25** ± 0.9	80* ± 2.1
2207	55.8	76** ± 4.1	26** ± 2.3	34	42	32** ± 0.9
2366	63.1	88	26** ± 2.5	30	24** ± 1.3	58* ± 2.1
2370	57.5	105	24** ± 1.1	23** ± 2.1	33.75	29.75** ± 0.5
2407	87.7	97	38	39	0** ± 0	100* ± 0
2410	69.8	103	32** ± 2.4	31	36.25	40.25
2412	67.3	107	25** ± 1.8	23** ± 1.4	44	44
2865	77.5	88	43	49	22** ± 0.9	66* ± 1.6
2866	75.1	90	41	46	13.75** ± 0.6	70* ± 2.9
2867	80.9	84** ± 4.9	46	55* ± 4.9	22.25** ± 1.0	65.75* ± 1.5
2868	92.3* ± 4.5	86** ± 5.3	30** ± 2.0	35	38	78* ± 2.7
2870	103* ± 5.0	109	53	48.6	12** ± 2.0	18** ± 1.1
2871	112* ± 3.9	68** ± 3.3	18** ± 0.9	27	50.25* ± 3.1	41.75
2872	96.6* ± 6.8	93	49	53	26** ± 0.9	70* ± 2.3
2873	77.3	88	25** ± 2.4	28	8** ± 0.4	70* ± 2.6
2874	113.2* ± 5.1	100	39	39	37.75	44
2876	73	85** ± 4.2	20** ± 1.8	23.5** ± 1.3	24** ± 1.6	60* ± 2.7
2877	86.5	112	47	42	34	53.75
2878	66.6	97	48	50	38	45.75
2879	121.1* ± 9.9	86** ± 3.9	28** ± 1.5	33	16** ± 0.4	76.25* ± 2.9
2881	55.7	91	23** ± 2.0	25** ± 2.0	36.25	55.75* ± 2.0
2882	100.9* ± 5.7	110	50	46	23.75** ± 1.3	65.75* ± 2.3
2883	77.9	92	26** ± 2.5	28	22** ± 0.7	36
2884	69.3	105	58* ± 3.7	55* ± 5.2	1.5** ± 0.3	72* ± 3.9
2885	81.1	88	29** ± 2.2	33	26** ± 0.6	72.25* ± 2.6
2886	66.9	97	43	44	38.25	56* ± 2.7
2887	52.3	87	37	43	40	58.25* ± 1.8
2888	58.7	68** ± 3.8	10** ± 0.4	15** ± 0.8	51.75* ± 3.8	32** ± 0.7
2890	45.5	65** ± 4.4	15** ± 1.1	23** ± 1.1	32	52.25
2892	62.8	93	23** ± 1.9	25** ± 1.3	18.25** ± 1.7	69.75* ± 3.5
2893	94.6* ± 7.0	101	46	46	31.75	53.75
2894	90.8* ± 5.9	75** ± 4.3	33** ± 2.9	44	28	50
2896	82.4	106	32** ± 1.8	30	47.75* ± 2.8	45.75
2898	121.9* ± 9.1	92	52	57* ± 4.7	28	59.75* ± 4.1
2899	103* ± 7.3	110	38	35	14** ± 0.4	72* ± 3.2
2900	90.5* ± 6.9	98	39	40	24** ± 1.1	52
2901	114.2* ± 5.8	102	55	54	24.25** ± 0.8	68* ± 1.5
2903	118.1* ± 10.1	94	47	50	0** ± 0	65.75* ± 2.8
2904	68.7	112	46	41	12** ± 1.2	72* ± 3.3
2905	77.6	76** ± 3.5	35	46	14** ± 0.4	78* ± 3.2
2908	42.1	60** ± 4.6	10** ± 0.6	17** ± 1.2	58* ± 3.3	34** ± 0.8
Kiršinai	61.1	75** ± 5.1	17** ± 0.5	23** ± 1.4	75.25* ± 3.6	12.25** ± 0.3
Liepsna	70	70** ± 3.8	23** ± 0.9	33	70* ± 4.5	42
Radviliai	91.3* ± 3.9	103	27** ± 0.7	26	44	32.25** ± 1.8
Sadūnai	69.9	111	47	42	32	56* ± 2.5
Vyliai	79.3	91	13** ± 0.6	14** ± 0.8	54* ± 2.3	43.75

*significantly higher than the standard Vytis $P \leq 0.05$ (Dunnett's test); **significantly lower than the standard Vytis $P \leq 0.05$ (Dunnett's test); FH – flower heads per plant; F/FH – florets per flower head; SS – seed set; GR – germination rate; HS – hard seeds

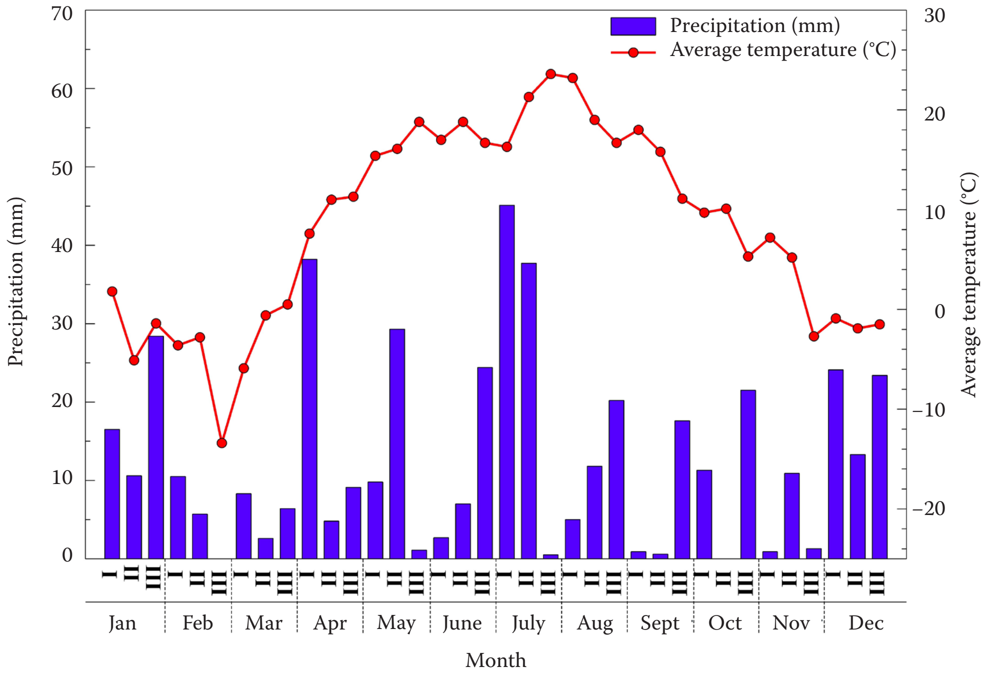

Figure 4. The meteorological conditions (air temperature and precipitation) at the Dotnuva site in 2018

extremely dry and hot. This month, the precipitation was only 37 mm, while the CN is 73.4 mm. In the meantime, the average temperature reached 19.5 °C and was 4.6 °C higher than the climatological norm. The high temperatures and water deficit was unfavourable for the plants' vegetation and yield formation. There were no changes in the early autumn either. September was very hot, sunny and windy. This month, the average temperature reached 15 °C and was 3.2 °C higher than the CN. In the meantime, the precipitation was very low and only reached 37.2% of the CN (51.3 mm). The average temperature in October was 8.3 °C and 1.4 °C higher than the CN. This month, the precipitation reached 32.8 mm. Due to lower temperature, evaporation was lower, therefore, the soil moisture became normal at the end of October.

DISCUSSION

Vleugels et al. (2019) and Dolferus et al. (2011) have reported that a low seed yield may be caused by several mechanisms, such as meiotic abnormalities, ovule abortion or poor pollination, as noted earlier by Bender (1999). However, Vleugels et al. (2015, 2016) and Amdahl et al. (2017) did not find a significant relationship between the seed yield and the flower pigmentation intensity. At the same time, Vanommeslaeghe et al. (2018) did not find that the dimension of the corolla tube correlates to an attractiveness to pollinators. These findings show that attempts were made to determine universal traits to predict the seed yield and its quality. However, there are not enough studies on the seed yield of wild red clover populations and its plasticity in agriculture areas. Research conducted by Vleugels et al. (2016) and Amdahl et al. (2016, 2017) on the seed yield and its components was carried out by analysing red clover cultivars only. A wild red clover accession collected in Andorra was used for the parentage analysis to improve the seed yield by Vleugels et al. 2014. However, there have been no reports of the use of such wild populations to breed new cultivars.

Due to the fact that wild red clover blooms about 2 weeks earlier than the earliest cultivars, it is possible to harvest seeds twice per season. The first seed yield depends on the climatic conditions and the soil moisture, while the second yield depends more on the plant's ability to regrow after the first cut (Loucks et al. 2018; Scotton 2018). Therefore, it is likely that the seed yield of the second harvest will always be lower than that of the first. However, our study showed that this may not be the case for all the populations, and some of them can produce a higher yield during the second harvest.

There are two main types of red clover which are differentiated by the growth rate, single-cut (commonly known as "mammoth") and double-cut (commonly known as "medium"). It is known that the single-cut red clover requires a longer day's length to bloom than the double-cut clover. Another distinctive feature is that the single-cut red clover is low growing and does not flower or produce stems in the seeding year (Bowley et al. 1984; Loucks et al. 2018). Double-cut red clover is more commonly grown, as it has a greater shoot biomass (Loucks et al. 2018). This assessment was later abandoned due to the difficulty in identifying the correct type. We found a population (accession 2871) that flowers early despite its low rate of green matter. The accession (2871) produces a high number of flower heads and intense colour. These are important traits to look for in a prototype for breeding ornamental cultivars.

Meanwhile, the most important indicator of increased seed yield in a breeding programme was the SN/FH. The same tendency was identified by Vleugels et al. (2016), where the seed number per ripened flower head (SN/rFH) and the number of rFH played the most important role in breeding to increase the seed production potential. We can confirm that tetraploids produce a lower number of seeds per flower head. Similar to the result obtained by Vleugels et al. (2016), we did not determine that tetraploids had fewer ripe flower heads per plant. Basically, the number of FH and F/FH were similar to those of diploids. Vleugels et al. (2016) reported that the seed production potential in diploid red clover could be raised by increasing the rFH and SN/rFH. The number of florets per flower head and the flower colour was similar in the diploids and tetraploids (Vleugels et al. 2016).

Red clover is species specified as having a large water demand. It has been reported that the water deficit impacts the shoot and inflorescence formation in the red clover (Staniak 2019). During this stage, drought stress effects on the dry matter yield (DMY) reduction up to 34%. Meanwhile, at flowering stage, it contributes to a low quantity of nectar as well as poor pollination (Staniak 2019). Even more, the water deficit affects the metabolic processes and reduces the biosynthesis precursors for the seed reserve (Sehgal et al. 2018). As a result, the seeds' quality decreases. They become smaller with a lower weight of the 1000 seed weight and have a higher number of hard seeds per accession. This affects the germination rate and vigour as well.

Loucks et al. (2018) reported that red clover populations with distinct growth habits could have different strategies for drought tolerance or survival. We have noticed that wild red clover is able to regrow well and tends to branch out more than the other cultivars after the first cut. *Inter alia*, the capacity to survive periods of moisture stress could be related to the maintenance of the viable meristematic tissue in the red clover crown (Loucks et al. 2018). It is assumed that some of the wild red clover accessions are more adapted to survive under abiotic stress, such as water deficit than their domesticated progenies.

CONCLUSION

The most important characteristic influencing the final seed yield was the SN/FH (the power was 0.906 in the first yield and 0.727 in the second yield). It was found that the seed characteristic rates between the first and the second harvest may differ significantly, as well as between the first and second yield. Three populations, 2898, 2899 and 2177, were distinguished by the highest seed yield, but differed slightly from Vytis, and the TSW of those populations was lower than that of the other cultivars. During the drought stress, the wild red clover plants were able to regrow and produce a second seed yield per season. However, the yield was lower during the second harvest. Also, it was found that the seeds were smaller during the second harvest, as a result, the 1000 seed weight was lower in most accessions. In terms of the phenotypic traits, the GR and hard seeds (HS) were negatively correlated and did not differ significantly for the final harvest forecast, but were valuable for the other seed rates. We identified that accessions 2177, 2871, 2876, 2885 and 2899 could be used to create a further cultivar prototype based on the seed yield and its components. Finally, the results of this study show that it is still suitable to search for and collect breeding material in the natural environment.

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Received: November 18, 2019
Accepted: June 24, 2020